Potential ameliorators of aflatoxicosis in weanling/growing swine.

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Potential Ameliorators of Aflatoxicosis in Weanling/Growing Swine[1,2]

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ABSTRACT: Three trials using 207 crossbred pigs were conducted to evaluate the effects of aflatoxin-contaminated corn on young pigs and to determine whether several nutritional and nonnutritional dietary amendments would benefit performance or health in situations of aflatoxin B_1 contamination. In Trial 1 using 90 pigs in a 49-d trial, linear ($P < .01$) and quadratic ($P < .05$) decreases in ADG were observed with increasing (0, 420, 840 ppb) dietary aflatoxin level. This growth decrease was associated with linear reductions ($P < .01$) in ADFI and decreases in gain/feed. Serum indicators of protein synthetic capabilities and of liver damage were also adversely affected. Improvements in growth rate for the total trial period in the presence of 840 ppb of aflatoxin were obtained with the addition of the 2 ppm of folic acid ($P < .05$) or .5% hydrated sodium calcium aluminosilicate (HSCA) ($P < .01$); the magnitude of improvement was greater for the HSCA. The addition of HSCA to the contaminated diet also restored the serum clinical chemistry profile to that exhibited by pigs fed the diet without contaminated corn. The addition of .6 ppm of Se to a basal diet containing .3 ppm of Se was generally without effect. In Trial 2, 63 pigs were used in a 42-d trial to further assess the effectiveness of both folic acid and HSCA, as well as of two sodium bentonites, in reducing the effects of aflatoxin from naturally contaminated diets (800 ppb of aflatoxin). Folic acid had no positive effect in this trial, but HSCA improved ADG ($P < .01$) and all clinical chemistry indicators that had been negatively affected by the contaminated diet ($P < .05$). Both sodium bentonites provided as much improvement in ADG ($P < .01$) as the HSCA and also improved all clinical chemistry indicators that had been adversely affected by the aflatoxin ($P < .05$). In Trial 3 using 54 pigs in a 42-d trial, linear ($P < .05$) and quadratic ($P < .025$) improvements in ADG and ADFI and linear ($P < .05$) improvements in those serum clinical chemistry indicators adversely affected by the contaminated diet were observed with additions of .25, .50, and .75% sodium bentonite to a diet naturally contaminated with 800 ppb of aflatoxin. There was no benefit to including more than .50% sodium bentonite to the contaminated diet. In situations of aflatoxin contamination, dietary alterations or additions can be made to diminish the adverse effects of aflatoxin.

Key Words: Pigs, Folic Acid, Selenium, Aflatoxins, Clay

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Introduction

One-quarter of the world's food crops may be affected by mycotoxins, which are the toxic metabolites of fungi growing on cereal grains that are produced during growth, harvest, transportation, or storage of the grains (Pier et al., 1980; CAST, 1989). Although an occasional animal death may be associated with the consumption of feeds containing an individual toxin, the greatest economic losses due to mycotoxins are reduced productivity, increased disease incidence because of immune suppression, subtle but chronic damage to vital organs and tissues, and interference with reproductive capacities (CAST, 1989).

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[2]Mention of a trade name does not constitute a guarantee or warranty by VPI&SU and does not imply its approval to the exclusion of other products that may be suitable.

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Aflatoxins are the specific mycotoxins produced by some strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Climatic conditions conducive to the growth of *Aspergillus flavus* exist in portions of the southeastern United States almost every year. Aflatoxins damage the liver, decrease milk and egg production, and suppress immunity in animals that consume low dietary concentrations. Nichols (1983) estimated the losses incurred in 1980 by hog producers in the southeastern United States from the use of aflatoxin-contaminated feeds at $100 million.

After Phillips et al. (1988) reported success in restoring portions of the lost growth rate associated with aflatoxin ingestion in poultry via additions of a hydrated sodium calcium aluminosilicate (**HSCA**) to the diet, we began a series of trials that addressed both nutritional modifications of the diet and the addition of various clays to the diet to ameliorate the adverse effects that aflatoxins have on swine performance. These studies were conducted to characterize the effects of feeding aflatoxin-infected corn on the performance, immunocompetence, and blood chemistry of weanling pigs and to evaluate potential ameliorators.

Table 1. Percentage composition of basal diets

Ingredient	Trial[a] 1	2	3
Ground corn	70.00	68.73	68.70
Soybean oil	—	1.00[b]	1.00[c]
Soybean meal (48% CP)	26.60	26.90	26.90
Dicalcium phosphate	1.20	1.20	1.20
Ground limestone	1.00	1.00	1.00
Vitamin premix	.25[d]	.25[e]	.25[d]
Salt	.40	.40	.40
Trace mineral premix[f]	.05	—	.05
Antibacterial[g]	.50	.50	.50
Antioxidant[h]	—	.02	—
Calculated composition, %			
Crude protein	18.85	18.96	18.96
Lysine	1.01	1.02	1.02
Ca	.77	.81	.81
P	.59	.65	.65

[a]Replacement of uncontaminated corn with appropriate amount of contaminated corn was performed to achieve the desired treatment contamination level. Dietary treatment amendments in each study were added at the expense of the uncontaminated corn.
[b]Food-grade soybean oil.
[c]Feed-grade soybean oil, degummed, stabilized with ethoxyquin; Occo Brand, Oelwein, IA 50662.
[d]Supplied per kilogram of diet: 4.34 mg of riboflavin, 22 mg of pantothenic acid, 22 mg of niacin, 22 μg of vitamin B_{12}, 440 mg of choline chloride, 4,409 IU of vitamin A, 441 IU of vitamin D, 11 IU of vitamin E, 1,102 μg of vitamin K (as menadione sodium bisulfate complex), and .1 mg of Se.
[e]Supplied per kilogram of diet: 11,000 IU of vitamin A, 550 IU of vitamin D_3, 44 IU of vitamin E, 220 μg of biotin, 6.6 mg of riboflavin, 22 mg of D-pantothenic acid, 28 mg of niacin, 33 μg of vitamin B_{12}, 5.5 mg of vitamin K (as menadione dimethylpyrimidinol bisulfite), 670 mg of choline, 125 mg of Zn, 125 mg of Fe, 40 mg of Mn, 10 mg of Cu, 1.25 mg of I, and .3 mg of Se.
[f]Contained 20% Zn, 10% Fe, 5.5% Mn, 1.1% Cu, and .15% I.
[g]Supplied per kilogram of diet: 110 mg of chlortetracycline, 110 mg of sulfamethazine, and 50 mg of procaine penicillin.
[h]Supplied 133.3 mg of ethoxyquin/kg of diet.

Experimental Procedures

Corn naturally contaminated with approximately 1,200 ppb of aflatoxin B_1 was obtained from a grain producer in southeast Virginia for Trial 1. The use of naturally contaminated corn was employed to mimic situations that occur in routine production. Ninety crossbred pigs (mean initial weight of 10.6 kg) were weaned at approximately 37 d of age and were randomly assigned from outcome groups based on litter, sex, and body weight to one of the following diets: 1) 0 ppb of aflatoxin, 2) 420 ppb of aflatoxin, 3) 840 ppb of aflatoxin, 4) 0 ppb of aflatoxin plus .6 ppm of Se from sodium selenite, 5) 420 ppb of aflatoxin plus .6 ppm of Se, 6) 840 ppb of aflatoxin plus .6 ppm of Se, 7) 0 ppb of aflatoxin plus 2 ppm of folic acid (**FA**), 8) 840 ppb of aflatoxin plus 2 ppm FA, 9) 0 ppb of aflatoxin plus .5% HSCA (NovaSil, Engelhard Corp., Cleveland, OH), and 10) 840 ppb of aflatoxin plus .5% HSCA. The basal diet and all other non-Se treatment diets were amended with sodium selenite to provide a total of .3 ppm of added Se. Dietary aflatoxin levels were obtained by varying the amount of contaminated and uncontaminated corn in the diet. Pigs were allowed ad libitum access to a 1.01% lysine corn-soybean meal diet that contained 70% corn (Table 1). A total of three replicates of three pigs/pen were used. Pen size was .9 m × 1.2 m. Feed was offered in a three-hole feeder and water was supplied by a nipple waterer. Nursery temperature was set initially at 27° C and reduced weekly to maintain a zone of thermoneutrality consistent with the needs of the smallest pigs. The trial length was 49 d.

Pigs were fed their respective diets for 14 d before an initial i.m. injection that contained the antigens concanavalin A and ovalbumin (Blodgett et al., 1986). Pigs were re-injected on d 38 of the experiment. Blood samples were obtained via vena cava puncture on d 14 and 38 before injection and on d 28 and 49 for assessment of immunoresponsiveness to the injected antigens. In addition, clinical chemistry analyses (total bilirubin, blood urea N, glucose, albumin, total serum protein, gamma glutamyl transferase [**GGT**], aspartate aminotransferase [**AST**], alkaline phosphatase [**ALP**], HCO_3, anion balance, Ca, P, Mg, Na, K, Cl) were conducted on the blood samples obtained on d 49. To estimate cell-mediated immune responsiveness, phytohemagglutinin was injected intradermally on the distal abdominal region on d 49 according to the procedures of Blodgett et al. (1986) and on d 50 the dermal response was measured with a constant tension micrometer as an estimate of T-cell responsiveness.

For Trial 2, corn naturally contaminated with approximately 2,300 ppb of aflatoxin B_1 (Table 2) was obtained and 63 crossbred pigs (mean initial age and weight were 36 d and 9.1 kg, respectively; pigs had been weaned 7 d before study initiation) were used in a 42-d trial. The purpose of the trial was to assess FA, HSCA, and two sodium bentonites (**SB1** and **SB2**; trade names are Volclay powder and FD-181, respectively; products are montmorillonite clays marketed by the American Colloid Company, Arlington Heights, IL) as ameliorators of the chronic effects of aflatoxin ingestion. The pigs were randomly assigned from outcome groups based on litter, sex, and body weight to one of the following treatments: 1) 0 ppb of aflatoxin, 2) 800 ppb of aflatoxin by replacing appropriate quantities of clean corn with the contaminated corn, 3) as Treatment 2 plus 2 ppm FA, 4) as Treatment 2 plus .5% HSCA, 5) as Treatment 3 plus .5% HSCA, 6) as Treatment 2 plus .5% SB1, and 7) as Treatment 2 plus .5% SB2. The bentonites were chosen for use in this trial after laboratory tests with a variety of clays had shown them to have potential to bind aflatoxin. Three replicate pens of three pigs/pen were used per treatment. Pen size and nursery management were the same as in Trial 1. The basal diet was a 1.02% lysine, corn-soybean meal diet with 1% added soybean oil for dust suppression (Table 1). Blood samples were obtained via vena cava puncture on d 42 for clinical chemistry analyses.

The third trial was conducted with 54 crossbred pigs (mean initial age and weight were 37 d and 9.2 kg, respectively; pigs had been weaned 7 d before allotment) to examine the effect of inclusion rate of a clay on the growth and serum profile response of the pigs. A sodium bentonite from Trial 2 (SB2) was selected for dose titration. Pigs were randomly assigned from outcome groups based on litter, sex, and body weight to one of the following treatments: 1) 0 ppb of aflatoxin B_1, 2) 800 ppb of aflatoxin by replacing appropriate quantities of clean corn with the contaminated corn used in Trial 2, 3) as Treatment 2 plus .25% SB2, 4) as Treatment 2 plus .5% SB2, 5) as Treatment 2 plus .75% SB2, and 6) as Treatment 1 plus .5% SB2. The basal diet was a 1.02% lysine corn-soybean meal diet, with 1% soybean oil for dust suppression, which met or exceeded all NRC (1979) nutrient requirement estimates for pigs used in this study. Three replicate pens of three pigs per pen were used per treatment for the 42-d study; pen size and nursery management were the same as in the other trials. Blood samples were obtained via vena cava puncture on d 42 for clinical chemistry analyses.

Aflatoxin content of the corns utilized in these studies was determined by a high performance liquid chromatography procedure (Pons et al., 1980). Serum clinical chemistry profile analyses were obtained through the Virginia/Maryland Regional College of Veterinary Medicine, which used a Kodak Ektachem 700 Analyzer for analysis. Mineral analysis of the corn was determined by inductively coupled plasma emission spectrometry after dry-ashing. Iron binding capacities were determined using a procedure based on that employed by Persijn et al. (1971).

Table 2. Nutrient composition (as-fed basis) of two corn sources used in Trials 2 and 3

	Corn	
Item	Uncontaminated	Contaminated
Aflatoxin B_1, ppb	0	2,300
Gross energy, kcal/kg	3,826	3,726
Ether extract, %	5.66	4.99
Crude protein, %	9.9	8.4
P, %	.27	.42
Ca, %	.01	.02
K, %	.36	.46
Na, %	.01	.02
Mg, %	.09	.16
Cu, ppm	2.8	4.7
Fe, ppm	33.3	114.0
Mn, ppm	6.8	13.4
Zn, ppm	29.0	43.7

Data from all trials were analyzed using the GLM procedure of SAS (1986). Orthogonal and preplanned nonorthogonal contrasts were used to compare treatment means. When appropriate, linear and quadratic components of the treatment effects were determined. Pen served as the experimental unit. Least squares means are reported.

Results and Discussion

In Trial 1, one pig on dietary Treatment 8 (the contaminated diet amended with 2 ppm FA) died after 31 d on test. Liver lesions exhibited by that pig were consistent with those commonly observed for aflatoxicosis. Before death the pig exhibited no overt signs of toxicity and growth performance was greater than that observed for many other pigs in the study. Daily gain in this trial was linearly decreased ($P < .01$) with increasing level of aflatoxin in the diet (Table 3). During the initial 28-d period, the intermediate level of aflatoxin (420 ppb, Diet 2) resulted in an intermediate level of daily gain. However, in the final 20-d period, pigs fed that diet gained at a rate comparable to pigs fed the control diet without aflatoxin. Improvements in growth were observed when HSCA ($P < .05$) was added to the diet in Period 1 and due to Se ($P < .05$), FA ($P < .05$), and HSCA ($P < .01$), dietary additions in Period 2. For the total trial period, pigs fed the contaminated corn diet with HSCA added attained weight gains almost identical to those of the control pigs fed the uncontaminated diet. Pigs fed the FA-supplemented diet exhibited a 40% recovery of the daily weight gain differential ($P < .05$)

Table 3. Performance of nursery pigs fed graded levels of aflatoxin-contaminated corn in Trial 1[a]

Item	Diet:	Basal			Basal + .6 ppm of Se			Basal + 2 ppm FA		Basal + .5% HSCA		SEM
	Aflatoxin, ppb:	0	420	840	0	420	840	0	840	0	840	
BW, kg												
Initial		10.6	10.5	10.6	10.6	10.8	10.6	10.5	10.5	10.6	10.8	.16
Intermediate[bgj]		23.7	20.4	16.5	23.1	20.4	15.8	24.1	18.2	23.7	21.3	.87
Final[bcfhj]		35.5	32.4	23.9	36.0	33.1	25.3	36.6	28.3	37.0	34.1	1.30
ADG, kg												
Period 1[bjg]		.47	.35	.21	.45	.34	.19	.49	.28	.47	.37	.030
Period 2[bdefhj]		.59	.60	.37	.65	.64	.48	.62	.50	.66	.64	.035
Overall[bcfhj]		.52	.46	.28	.53	.47	.31	.54	.37	.55	.48	.027
ADFI, kg												
Period 1[bg]		.79	.69	.52	.79	.69	.47	.83	.64	.83	.76	.051
Period 2[bhj]		1.61	1.32	.84	1.69	1.43	.98	1.66	1.10	1.72	1.73	.089
Overall[bhj]		1.13	.95	.67	1.16	1.00	.68	1.18	.83	1.20	1.17	.060
Gain/feed												
Period 1[bi]		.60	.51	.30	.56	.50	.39	.59	.43	.57	.48	.071
Period 2[bj]		.37	.45	.43	.38	.45	.48	.38	.45	.38	.37	.021
Overall		.46	.48	.37	.45	.47	.41	.46	.45	.46	.41	.027
Corrected[bki]		.58	.52	.37	.57	.52	.44	.57	.48	.60	.47	.038

[a]Period 1 covers initial 28 d; Period 2 covers final 20 d. Each mean represents three pens of three pigs/pen with the exception of column 8, which represents two pens of three pigs/pen and one pen of two pigs/pen. FA = folic acid; HSCA = hydrated sodium calcium aluminosilicate.
[b]Linear effect of aflatoxin, $P < .01$.
[c,d]Quadratic effect of aflatoxin, $P < .05$, .01, respectively.
[e]Effect of Se, $P < .05$.
[f]Folic acid effect, $P < .05$.
[g,h] HSCA effect, $P < .05$, .01, respectively.
[i,j] HSCA × aflatoxin interaction, $P < .10$, .05, respectively.
[k]Folic acid × aflatoxin interaction, $P < .05$.

between the unamended diets with 0 and 840 ppb of aflatoxins. Previous research with Se (Davila et al., 1983), FA (Combs and Harrison, 1982; Campbell et al., 1987), and HSCA (Colvin et al., 1989; Harvey et al., 1989) has demonstrated various degrees of improvement in growth performance in situations of aflatoxin contamination. The magnitude of improvement in the earlier trials suggested that the greatest response would be seen with the HSCA. That was indeed demonstrated in this trial, which allowed direct comparison of all of these treatments.

The majority of the differences in weight gain were due to alterations in feed intake. A problem existed in the interpretation of gain/feed values because the pigs fed the unamended diet with 420 ppb of aflatoxin had one of the more desirable values for the total trial. Several factors contributed to this problem in interpretation. The first factor was that the nursery was maintained at an optimal temperature for the smallest pigs, which were those consuming diets with the contaminated corn. As the trial progressed the difference in body size between the treatment groups became greater, such that at the end of the trial the heaviest pigs were experiencing heat stress, which contributed to their elevated gain/feed values. Second, the unanticipated rank of gain/ feed values is affected very much by the widely divergent body weights at trial termination. When the gain/feed values are corrected to a final body weight similar to that of the lightest pigs (comparing gain/feed then over a common weight range rather than a common time length), the values are ranked more as one might expect. Additionally, it is known that moderate feed restriction can improve gain/feed values of pigs; in the present study, pigs fed Diet 2 containing 420 ppb of aflatoxin consumed about 84% of that consumed by the pigs fed the uncontaminated diet, a restriction that contributes to the elevation of feed/gain value over the expectation.

In instances of aflatoxicosis, several indices of liver function are affected. With liver damage, bilirubin increases, blood urea N decreases, proteins synthesized by the liver decrease, and hepatic enzymes (such as gamma glutamyl transferase and alkaline phosphatase) appear in greater concentrations in the blood (Duncan and Prasse, 1986). Harvey et al. (1988a) reported the results of a study evaluating dosage of aflatoxin and time of exposure that effectively illustrates the progression of changes in serum enzymes, organ weights, and several other measurements when pigs are exposed to aflatoxins. The magnitude of the effect is dependent on both the level of contamination and the length of time that pigs are exposed to the diet. The clinical chemistry analyses (Table 4) indicated that liver damage was occurring as a result of the increasing aflatoxin levels, especially the highest level, 840 ppb. Selenium, FA, and HSCA all improved some measure of liver function, but HSCA clearly provided the most significant improvements. Monovalent minerals, bicarbonate and anion gap are

all involved in, or indicators of, acid/base balance and ability to buffer acid/base loads. Although treatment effects were noted, the values for pigs on the various treatments fell within normal ranges (Pond and Houpt, 1978) and the effects were quantitatively smaller than the effects observed as indicators of liver function. A linear ($P < .01$) decline in cell-mediated immune responsiveness was evident with increasing aflatoxin level; HSCA restored cell-mediated responsiveness ($P < .01$).

Several other indices affected by aflatoxin in previous studies were also measured (Table 5). The anamnestic humoral response to the novel antigen concanavalin A was increased ($P < .05$) by aflatoxin consumption and by .6 ppm of Se addition above control Se levels. Although a similar increase in humoral immunity has been previously noted in chickens fed aflatoxin (Boonchuvit and Hamilton, 1975), most swine studies with aflatoxin have found either no change (Panangala et al., 1986) or a decreased response depending on the antigen used (Miller et al., 1978). The tendency of .9 ppm added Se to increase humoral immunity in swine has been previously observed (Blodgett et al., 1986). Complement was unaffected by aflatoxin, but levels were inexplicably increased by HSCA. Total serum iron increased with aflatoxin consumption. It is possible that the corn containing the aflatoxin was higher in iron than the uncontaminated corn, as was the case in Trial 2 (Table 2). Because the total iron-binding capacities were numerically similar, the synthesis of the beta-1 globulin, transferrin, was unaffected. Dietary aflatoxin concentrations greater than 2 ppm fed to pigs have decreased transferrin synthesis (Harvey et al., 1988b).

After the initial trial that confirmed the positive response of pigs to HSCA additions to diets containing 840 ppb of aflatoxin, two commercially available forms of sodium bentonite were evaluated in diets contaminated with 880 ppb of aflatoxin from naturally contaminated corn. The contaminated diet reduced growth rate by 36% (.23 kg/d) over the 42-d trial (Table 6). The addition of either SB1 and SB2 to the contaminated diet improved ADG ($P < .01$) over that obtained from the unamended, contaminated diet, as did the addition of the HSCA ($P < .01$). Although numerical improvements were observed in both feed intake and ADG with the addition of FA to the diet, the responses were not of the magnitude seen in Trial 1. In Trial 2, the gain/feed values illustrate no effect of aflatoxin contamination or dietary amendment on efficiency; the effects of dietary treatments on growth rate seem to be mediated entirely through alterations in feed intake. Twenty-eight days after the trial was

Table 4. Effect of diet on blood clinical chemistry values and response to phytohemagglutinin antigen in Trial 1[a]

Diet:	Basal			Basal + .6 ppm of Se			Basal + 2 ppm FA		Basal + .5% HSCA		
Item Aflatoxin, ppb:	0	420	840	0	420	840	0	840	0	840	SEM
TB ($\times 10^{-3}$), mg/dL[bfhjl]	100	96	299	84	124	82	58	173	82	117	62.7
BUN, mg/dL[klm]	19.4	14.3	13.2	12.8	17.9	16.4	17.3	17.2	18.5	17.2	1.56
Glucose, mg/dL[cj]	104	103	93	109	104	100	108	98	108	107	3.6
Albumin, g/dL[cjk]	3.8	3.7	3.4	3.7	3.7	3.4	3.8	3.6	3.8	3.8	.14
TP, g/dL	6.4	6.7	6.8	6.6	6.9	6.6	6.6	6.7	6.7	6.8	.16
GGT, IU/L[b]	25	38	42	26	33	35	19	31	19	32	6.5
AST, IU/L[cdjkl]	33	59	208	41	64	110	40	107	38	48	43.5
Ca, mg/dL	11.0	11.3	11.5	11.0	11.3	11.0	11.2	11.1	11.0	11.2	.14
Mg, mg/dL	2.3	2.4	2.4	2.3	2.5	2.4	2.3	2.4	2.4	2.4	.10
P, mg/dL[bdjk]	10.0	10.1	9.3	10.2	10.4	9.6	10.6	9.6	9.9	11.0	.41
Na, mEq/L[cdgjk]	144	144	141	147	147	143	144	143	144	147	1.4
K, mEq/L[efj]	5.6	5.9	5.7	6.0	6.5	5.6	6.0	5.9	6.1	6.3	.23
Cl, mEq/L[b]	103	103	100	103	102	102	101	101	102	102	.8
HCO_3, mEq/L[eijkm]	28.9	27.2	25.3	28.8	27.6	27.8	31.0	31.1	28.8	29.2	.88
AG, mEq/L[efil]	18.1	20.1	21.0	20.6	23.9	19.1	17.4	17.1	19.4	21.7	1.30
PHA, mm[cjk]	7.2	6.6	6.4	7.2	7.1	6.1	7.1	6.4	7.6	7.7	.24

[a]Values are for d 49 on test and represent the mean of three pens of three pigs/pen per treatment with the exception of column 8, which represents two pens of three pigs/pen and one pen of two pigs/pen. TB = total bilirubin; BUN = blood urea N; TP = total protein; AG = anion gap; PHA = phytohemagglutinin response; GGT = gamma glutamyl transferase; AST = aspartate aminotransferase.

[b,c]Linear effect of aflatoxin, $P < .05$, .01, respectively.

[d,e]Quadratic effect of aflatoxin, $P < .10$, .05, respectively.

[f,g]Effect of Se, $P < .10$, .05, respectively.

[h,i]Folic acid effect, $P < .10$, .05, respectively.

[j]HSCA effect, $P < .05$.

[k]HSCA × aflatoxin interaction, $P < .05$.

[l]Se × aflatoxin, $P < .05$.

[m]Folic acid × aflatoxin, $P < .05$.

Table 5. Serum antibody, iron, and selenium concentration of weanling pigs fed graded levels of aflatoxin-contaminated corn with various ameliorators in Trial 1[a]

Item	Diet:	Basal			Basal + .6 ppm of Se			Basal + 2 ppm FA		Basal + .5% HSCA		SEM
	Aflatoxin, ppb:	0	420	840	0	420	840	0	840	0	840	
Concanavalin A[b]												
Day 28[h]		.8	1.2	1.9	1.8	2.7	1.1	1.9	1.5	1.6	2.2	.43
Day 49[ce]		2.7	2.5	4.3	3.4	4.4	4.2	3.1	3.7	3.0	4.0	.46
Ovalbumin[b]												
Day 28		.2	.8	.5	.5	.6	.4	.8	1.2	.8	.6	.32
Day 49		5.7	6.2	7.1	5.2	7.8	6.2	6.8	6.3	5.2	6.4	.69
Complement, units/mL[f]		30.5	50.3	46.7	52.6	64.0	48.6	45.7	48.6	43.4	71.4	8.98
Total Fe, μg/dL[ceg]		147.9	202.7	247.0	138.6	129.9	170.1	—	—	152.1	185.7	22.0
Total unbound Fe binding capacity, μg/dL[cd]		323.5	327.4	272.4	344.3	369.2	289.5	—	—	348.8	317.2	20.9
Total Fe binding capacity, μg/dL[dgh]		471.4	530.1	519.4	482.9	499.0	459.7	—	—	500.9	502.9	16.8
Total Se, ppb[e]		105	113	103	125	115	139	—	—	—	—	5.8

[a]Least squares means; each mean represents three pens of three pigs/pen with the exception of column 8, which represents two pens of three pigs/pen and one pen of two pigs/pen. FA = folic acid; HSCA = hydrated sodium calcium aluminosilicate.
[b]ELISA units and corresponding antibody titers: 0 = ≤ 1:50, 1 = 1:100, 2 = 1:200, 3 = 1:400, 4 = 1:800, 5 = 1:1,600, 6 = 1:3,200, 7 = 1:6,400, 8 = 1:12,800.
[c]Linear effect of aflatoxin, $P < .05$.
[d]Quadratic effect of aflatoxin, $P < .05$.
[e]Selenium effect, $P < .05$.
[f]HSCA effect, $P < .05$.
[g]HSCA × aflatoxin interaction, $P < .05$.
[h]Selenium × aflatoxin, $P < .05$.

initiated, one pig given the contaminated dietary treatment with 2 ppm FA added died. The necropsy report indicated liver lesions consistent with aflatoxicosis.

The indicators of nitrogen utilization and protein synthetic capabilities such as blood urea N ($P < .05$), total protein, and albumin ($P < .05$) levels in the blood were decreased for pigs fed the contaminated diet. Certain serum enzymes such as AST, ALP, and GGT that are usually elevated in aflatoxicosis and that indicate damage to hepatocytes tended to be elevated, but only ALP differences were of sufficient degree to be significant ($P < .05$). This illustrates the need for a clinical chemistry profile rather than an

Table 6. Performance and serum clinical chemistry of weanling pigs fed aflatoxin-contaminated corn with various ameliorators in Trial 2[a]

Item	Ameliorator:	—	—	FA	HSCA	FA/HSCA	SB1	SB2	SEM
	Aflatoxin, ppb:	0	800	800	800	800	800	800	
BW, kg									
Initial		9.1	9.1	9.3	9.0	9.1	9.1	8.9	.12
Final[cegi]		36.2	26.5	27.2	34.3	34.4	34.9	35.1	.73
ADG, kg[cegi]		.64	.41	.43	.60	.60	.61	.62	.017
ADFI, kg[cegi]		1.32	.82	.88	1.23	1.24	1.21	1.33	.023
Gain/feed		.49	.50	.48	.49	.48	.51	.47	.017
Corrected		.50	.50	.54	.51	.51	.53	.50	.033
Serum chemistry									
BUN, mg/dL[bdfh]		14.4	9.9	10.3	15.1	14.9	15.7	13.2	.80
Albumin, g/dL[bdfh]		3.9	3.3	3.5	3.9	3.8	4.0	4.0	.10
TP, g/dL		6.4	6.0	6.3	6.4	6.2	6.4	6.4	.15
AST, IU/L		58	83	83	61	64	58	67	8.7
ALP, IU/L[bdfh]		180	262	248	182	201	194	199	20.9
GGT, IU/L		50	68	79	52	58	59	58	5.5

[a]Length of trial was 42 d. Each mean represents three pens of three pigs/pen with the exception of column 3, which represents two pens of three pigs/pen and one pen of two pigs/pen. FA = 2 ppm of folic acid, HSCA = .5% hydrated sodium calcium aluminosilicate; SB1 = .5% sodium bentonite 1 (volclay), SB2 = .5% sodium bentonite 2 (FD-181). BUN = blood urea N; TP = total protein; AST = aspartate amino transferase; ALP = alkaline phosphatase; GGT = gamma glutamyl transferase.
[b,c]Aflatoxin effect (column 1 vs column 2, $P < .05, .001$, respectively).
[d,e]HSCA effect (column 4 vs column 2, $P < .05, .01$, respectively).
[f,g]SB1 effect (column 6 vs column 2, $P < .05, .01$, respectively).
[h,i]SB2 effect (column 7 vs column 2, $P < .05, .001$, respectively).

Table 7. Performance and serum clinical chemistry of weanling pigs fed aflatoxin-contaminated diets with graded levels of sodium bentonite in Trial 3[a]

Item	Sodium bentonite, %:	0	.50	0	.25	.50	.75	SEM
	Aflatoxin, ppb:	0	0	800	800	800	800	
BW, kg								
Initial		9.2	9.4	9.2	9.2	9.2	9.1	.10
Final[cdf]		32.4	32.6	26.2	30.1	31.0	31.0	.73
ADG, kg[cdf]		.58	.58	.42	.52	.55	.55	.017
ADFI, kg[cdf]		1.26	1.33	.93	1.19	1.23	1.27	.041
Gain/feed		.46	.44	.46	.44	.44	.43	.022
Corrected[g]		.51	.44	.46	.44	.47	.48	.030
Serum chemistry								
BUN, mg/dL[dh]		14.6	18.1	11.7	13.9	15.7	15.2	1.23
Albumin, g/dL[be]		4.0	4.1	3.3	3.7	4.1	4.2	.20
TP, g/dL[bd]		6.1	5.9	5.6	5.7	6.2	6.3	.19
AST, IU/L		75	74	74	63	65	70	9.2
ALP, IU/L		203	171	268	231	213	229	22.7
GGT, IU/L[bd]		49	41	84	55	54	53	7.8

[a]Product used was FD-181. BUN = blood urea N; TP = total protein; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma glutamyl transferase.
[b,c]Aflatoxin effect (column 1 vs column 3, $P < .05$ and .01, respectively).
[d,e]Sodium bentonite linear response in aflatoxin-contaminated diets ($P < .05$ and .01, respectively).
[f]Sodium bentonite quadratic response in aflatoxin-contaminated diets ($P < .025$).
[g]Compares gain/feed values to a similar BW as was obtained by pigs fed the unamended contaminated diet.
[h]Column 1 vs column 2, $P < .07$.

assessment of a single indicator, because no single clinical chemistry indicator always responds. Aflatoxicosis is indicated by changes in the overall profile. The clinical profile in Table 6 illustrates these general changes due to aflatoxin ingestion. The general picture was one of adverse effects on protein synthetic capabilities as indicated by reductions in blood urea N, albumin and total protein and on hepatocytes as indicated by increases in AST, ALP, and GGT. These adverse effects were countered to varying degrees by the dietary amendments. Although the numerical improvements were not always significant at the $P < .05$ level of probability, the use of the bentonites and HSCA in the contaminated diets provided a clinical profile that more resembled that of the pigs fed the uncontaminated diet.

In Trial 3 the inclusion rate was evaluated in a dose-response trial using one of the sodium bentonites that had demonstrated efficacy in Trial 2. The response was a typical dose relationship response (Table 7). The anticipated reduction in growth rate due to aflatoxin contamination of the diet (27%, .16 kg/d) was reversed in a linear ($P < .05$) and quadratic ($P < .025$) manner with additions of the sodium bentonite to the contaminated diet. The addition of the first increment of sodium bentonite gave the greatest response, the second increment gave a moderate response, and the final increment gave little additional response. The growth rate improved through changes in feed intake with no significant effects on gain/feed. The clinical chemistry profile mirrored the responses observed in ADG and ADFI. These results indicate that an inclusion rate greater than .5% provides no additional benefit under conditions similar to those in this trial. However, a lower inclusion rate may be sufficient to maximize the response at lower levels of aflatoxin contamination.

Several precautions should be considered before adding clays to the diet. All clays should not be assumed to be the same; not all clays were satisfactory based on our original laboratory screening. An additional precaution is that some clays can bind drugs in feed. Many of the clays have GRAS (Generally Recognized As Safe) status for inclusion in unmedicated feeds as binders, pelleting aids, or anticaking agents when used in accordance with good manufacturing practices and when the limitations listed in the Association of American Feed Control Officials manual (AAFCO, 1990) are not exceeded. The AAFCO (1990) manual further states that the clays that they list "are not prohibited in medicated feeds for the same purpose and at the same level when it can be demonstrated that they do not interfere with the analysis of the drug by acceptable methods. It is the manufacturer's responsibility to determine and submit adequate data to support the conclusion that interference does not occur before using these products in a feed containing a drug." None of the clays is currently approved by the Food and Drug Administration for their ability to adsorb aflatoxins.

Implications

Aflatoxins are a problem that many producers and feed manufacturers have to deal with at some time. A number of dietary modifications (i.e., addition of certain clays, enhanced nutrient levels) can be employed in conjunction with proper purchasing,

storage, and handling of grains to limit the adverse effects of aflatoxin contamination. The identification of several clays that have the ability to limit the adverse effects of aflatoxin contamination may provide producers and feed manufacturers with alternatives. Selection of the specific clay to be used can then be based on location, cost, handling characteristics, or other properties unique to the particular product.

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